Technical Tips Online, Vol. 2, 1997

A PCR method for distinguishing cells from mouse strains 129 and C57BL/6 for gene knockout studies

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Keywords: Polymerase chain reaction

Most embryonic stem (ES) cell lines and host embryos used in gene knockout studies are from 129 and C57BL/6 strains, respectively. There has been only a single method, isozyme analysis, to distinguish the 129 strain from the C57BL/6 strain (Ref. 1, 2, 3). Here, we report an alternative, simple and sensitive PCR method to distinguish the 129/Ola strain from the C57BL/6J strain. The method is useful for gene knockout studies in specific common circumstances. Knockouts of many genes of interest result in embryonic lethality in homozygotes, thus preventing the investigation of the role of gene expression in adult tissues. One powerful approach to analyze the role of the gene product and cause(s) of embryonic lethality is to create a chimeric mouse using homozygous, negative ES cells in which both alleles have been targeted (for example, see Ref. 2, 3). Investigating the development of such chimeric mice requires determination of the contribution of 129 mutant cells to various tissues (Ref. 2, 3). Also, mutations that block gametogenesis or yield defective gametes may not be transmitted by a chimera but could be studied in the chimera by using PCR to distinguish gametes derived from either 129 or C57BL/6 strains.

Our method for distinguishing DNA from the two mouse strains, 129/Ola and C57BL/6J, is based on microsatellite polymorphism. Microsatellites are genomic elements composed of tandem repeats of short nucleotide sequences and the number of repeats in the sequence varies among different strains of mice. From about 2000 polymorphic loci placed on mouse chromosomes 1–6, we selected 15 loci where there are known size differences of microsatellites between C57BL/6J and seven other strains. Information about strain 129 was not available from the Mouse Genome Database (MGD, The Jackson Laboratory). PCR experiments were performed using primers [MapPairs™ (Research Genetics)] that amplify the 15 microsatellites and template DNAs from C57BL/6J and 129/Ola. The genomic DNA of 129/Ola was isolated from ES cells (BK4 cells, Ref. 4) and C57BL/6J DNA (The Jackson Laboratory) was obtained commercially. Amplification was performed in a volume of 50 μl containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.13 μM of each primer, 100 ng of template DNA, and 2.5 U Taq DNA polymerase (Boehringer Mannheim). The reaction conditions were 1 min at 95°C, 1 min at 50°C, 30 s at 72°C for 37 cycles. 10 μl of each PCR product was resolved on a 2% agarose gel [SeaKem LE (FMC BioProducts)]. Five out of 15 loci gave distinctive amplification with size differences between 129/Ola and C57BL/6J (Fig. 1a). The sequences of the primers from the five loci are listed (Table 1). The information on the primer sequences was obtained from the MGD. When these five primer pairs were tested further for PCR amplification of a mixture of 129/Ola and C57BL/6J DNAs, one of the primer pairs (MPC297) resulted in the clearest band pattern among the five primer pairs. Using MPC297, we obtained two distinctive bands with varying levels of band intensity depending on the amount of template DNA from each strain in the mixture (Fig. 1b, lanes 1–5). In addition, this primer pair was used to amplify template DNA from tails of chimeras developed from C57BL/6J blastocysts injected with 129/Ola ES cells. The amplification generated PCR products of two different sizes, each corresponding to 129/Ola and C57BL/6J, as expected (Fig. 1b, lane 6).

One recent report presented information on PCR-amplified microsatellites for 129/Sv and C57BL/6J, but these products were analyzed on denaturing polyacrylamide gels by autoradiography of the labeled PCR

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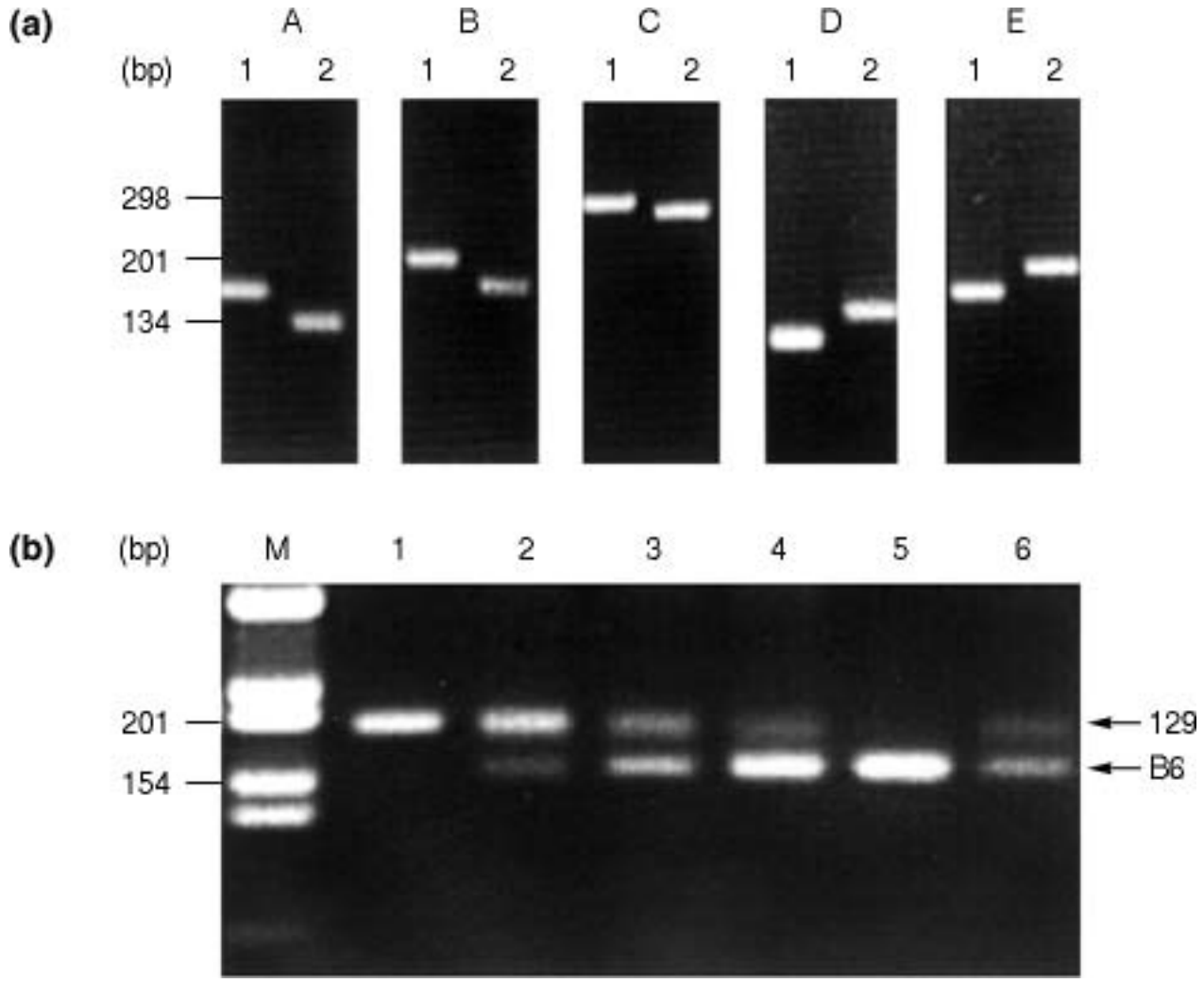

FIGURE 1. PCR amplification of 129/Ola and C57BL/6J DNAs with primers from microsatellite loci. (a) Genomic DNAs from 129/Ola (lane 1) and C57BL/6J (lane 2) were subjected to PCR amplification with five primer pairs: A, MT2991; B, MPC297; C, MT2333; D, A1110; E, M15. (b) Various amounts of 129/Ola and C57BL/6J DNAs were amplified by PCR with a primer pair, MPC297. Lane 1, 100 ng of 129/Ola DNA; lane 2, 75 ng of 129/Ola DNA and 25 ng of C57BL/6J DNA; lane 3, 50 ng of 129/Ola and 50 ng of C57BL/6J DNA; lane 4, 25 ng of 129/Ola DNA and 75 ng of C57BL/6J DNA; lane 5, 100 ng of C57BL/6J DNA; lane 6, 10 ng of genomic DNA from the tail of a chimera.

Table 1. PCR primers amplifying the products of different sizes between 129/Ola C57BL/6J

Locus (chromosome)	Primer Pair	Primer sequences
D Mit399 (1)	MT2991	5′-GTAGAGAATGATAAAGGACATCCTCC-3′
		5′-GGGAGGCTGAGATCTGTCAG-3′
		5′-TGAAAGTTCAGATGACCACACG-3′
D2Mit277 (2)	MT2333	5′-ATTCTGCATTCAAGCAAATTCA-3′
		5′-CTTGAAAGTAGTTGGTTGGTTGG-3′
D3Mit40 (3)	A1110	5′-CAGCTGGTCTAACTATCCCCC-3′
		5′-CCTTATTAAGTGCATGACCTTGC-3′
D4Mit12 (4)	M15	5′-GCTTGCTTTAGGAGTGTGCC-3′
		5′-TATTTGCTCTCCATTTCCCC-3′

products. [The microsatellite loci analyzed in the present report were not included in that study (Ref. 5).] Most microsatellite polymorphisms require NuSieve™ agarose gels (FMC BioProducts) or polyacrylamide gels with silver staining or autoradiography of denatured PCR products because of the small size differences between strains. An advantage of the present PCR method using these primer pairs is that the two distinctive PCR products can be resolved on a standard agarose gel. Thus, the method is fast and saves effort.

Acknowledgements

We thank Michael Seldin for advice on microsatellite information, Jimmy Spearow for helpful discussion and E. M. Eddy for providing chimera tails. This work was supported by NIH grants RO1 HD16580 (D.G.M.) and U54 HD29125 (D.G.M. and P.P.).

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Products Used

MapPairs(TM): MapPairs(TM) from Research Genetics
Taq DNA Polymerase: Taq DNA Polymerase from Boehringer Mannheim
Taq polymerase: Taq polymerase from Pharmacia
Taq polymerase: Taq polymerase from Bioline
Taq polymerase: Taq polymerase from Boehringer Mannheim
Taq polymerase: Taq polymerase from Boehringer Mannheim
Taq polymerase: Taq polymerase from Bioline
Taq DNA polymerase: Taq DNA polymerase from PE Applied Biosystems
Taq DNA Polymerase: Taq DNA Polymerase from Boehringer Mannheim
Taq DNA polymerase: Taq DNA polymerase from Life Technologies (Gibco BRL)
Taq DNA polymerase: Taq DNA polymerase from Promega Corporation
Taq DNA polymerase: Taq DNA polymerase from Stratagene
Taq DNA polymerase: Taq DNA polymerase from Takara Shuzo
Taq DNA polymerase: Taq DNA polymerase from Amersham Pharmacia Biotech
SeaKem LE: SeaKem LE from FMC BioProducts
agarose gel: agarose gel from Sigma
NuSieve: NuSieve from FMC BioProducts
agar: agar from Difco